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PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

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This paper attempts to define the relationship between the abnormal substances excreted in acute porphyria and the clinical manifestations of this disorder of porphyrin metabolism. During an attack of acute porphyria, patients usually excrete large quantities of porphobilinogen either alone or with certain porphyrins. The excretion of the porphyrins and porphobilinogen is usually in direct proportion to the severity of the symptoms, suggesting a causal relation, although Waldenström (1939) reported an authenticated case in which the patient did not pass uroporphyrin or porphobilinogen in the urine or bile during the attack, but did so on other occasions. Several authors have claimed that porphyrins may influence the placenta or uterus (Supniewski, 1927; Gunther, 1928; Reitlinger and Klee, 1928; Vannotti, 1937; Simici, 1938). Critical appraisal of these reports has led us to repeat this work using porphyrins of the kind known to be excreted in porphyria, which were obtained by improved methods of purification. The isolation of porphobilinogen in crystalline form (Westall, 1952) has for the first time allowed pharmacological testing of the pure substance, although Waldenström and Wendt (1938) and Prunty (1945) had injected partially purified porphobilinogen into rabbits.

METHODS

Animal Experiments.—Observations were made on blood pressure (recorded with a cannula in the carotid artery) and on the respiration of 13 cats and 10 rabbits (anaesthetized with chloralose (80 mg./kg.) by induction with ether) and of 1 pithed cat, 1 pithed viscera cat, and 1 decerebrate cat. Injections were made into the right femoral vein or the splenic vein. Stimulation of the distal end of the vagus, separated from the trunk in the neck, was with supramaximal 0.5 msec. pulses at 10 c./s.

Isolated Organs.—Experiments were also made on isolated strips of guinea-pig ileum, non-pregnant rabbit uterus, rabbit jejunum and ileum, or cat ileum, set up in Tyrode's solution at 34° C. Contractions were recorded on smoked paper by a frontal writing lever. Experiments with light irradiation were done with an electric bulb of 300 w. at 25 cm. from the tissue in the organ bath.

Drugs.—The porphyrins, with the exception of haematoporphyrin, had been isolated from biological material as the methyl esters. Before use the esters were hydrolysed with 7 N HCl for 36 hours, at room temperature, the excess of HCl being then removed in a vacuum desiccator over KOH. Haematoporphyrin was prepared and used as the dihydrochloride. Pure crystalline porphobilinogen (Westall, 1952) was used; the porphobilin was obtained by Mr. R. G. Westall as a by-product in the preparation of porphobilinogen.

Porphobilinogen was dissolved in a minimum volume of 0.1 N NH_4OH and then made up to the required volume with 0.9% saline. For the porphyrins and porphobilin M/7 sodium bicarbonate was used as the solvent.

RESULTS

Anaesthetized Cats and Rabbits.—Recordings were made of the direct effect of porphobilinogen—and, in one experiment, of uroporphyrin I—on the blood pressure, respiration and vascular responses of the treated animals to acetylcholine, histamine, nicotine, adrenaline, noradrenaline, and vagal stimulation. The amount of porphobilinogen injected (up to 100 $\mu\text{g./kg.}$) was limited by the amount available; but from the known rate of excretion in patients (40–160 mg./day), and from the fact that tests for plasma porphobilinogen sensitive to 1 $\mu\text{g./ml.}$ may be negative even at the height of an attack, it is likely that the blood levels obtained in our experiments were comparable to or even greater than those obtaining in acute porphyria. The only effect observed was an apparent potentiation by porphobilinogen of the response to adrenaline and noradrenaline in a few of the early

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experiments (Fig. 1). This apparent potentiation of adrenaline and noradrenaline could not, however, be repeated and its interpretation is complicated by the fact that considerable spontaneous fluctuations in sensitivity to these drugs may occur.

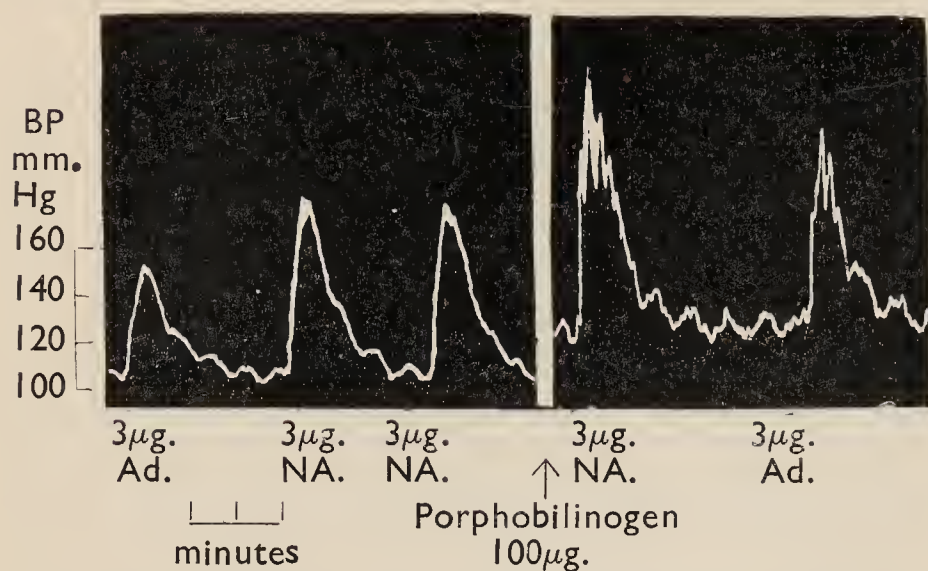


FIG. 1.—Cat.; chloralose; blood pressure recording; intravenous injections. Responses to 3 μ g. adrenaline and noradrenaline before and after 100 μ g. porphobilinogen.

Isolated Organs.—After obtaining records of spontaneous activity and tone, and of consistent responses to acetylcholine, histamine, adrenaline, and 5-hydroxytryptamine, the effect of adding porphyrins, porphobilinogen, and porphobilin to the preparation was investigated. With uropor-

phyrin I the effect of light irradiation was determined. A summary of these results is given in Table I.

The only significant responses were those to haematoporphyrin (1/8,000) and to porphobilinogen. The former produced a distinct waning contraction of guinea-pig ileum, followed by inactivity of intestine and a refractoriness—which became complete—to histamine and acetylcholine. Rabbit intestine was unaffected. Porphobilin produced a histamine-like contraction, sensitive to mepyramine but less so to atropine (Fig. 2); it was considered likely that the effect was due to contamination (c. 1 mg./g.) with histamine itself.

Test of Porphobilinogen on Unanaesthetized Rabbit.—10 mg. porphobilinogen was injected intravenously into a rabbit (2.2 kg.) with an external bile fistula. The animal showed no abnormal symptoms in the 3 days following the injection. There was a slight rise in the level of bile protoporphyrin during this period and a trace of uroporphyrin was noted in the urine several hours after injection. No porphobilinogen was found in the urine.

Test of Whole Urine from Patients with Acute Intermittent Porphyria.—As a final test, to cover the possibility that in porphyria some unidentified pharmacologically active substance was present, the effect of whole urine from patients with acute intermittent porphyria was tested on isolated tissues.

TABLE I
TESTS OF PORPHOBILINOGEN AND PORPHYRINS ON ISOLATED TISSUES

		Porphobilinogen	Uroporphyrin		Coproporphyrin		Haematoporphyrin	Porphobilinogen
			I	III	I	III		
Rabbit uterus	Drug concn.	1/40,000	1/10,000	1/40,000	1/40,000	1/40,000		
	Effect on spontaneous activity and tone	0	0	0	0	0		
	Effect on response to adrenaline	0	0	0	0	0		
Rabbit ileum or jejunum	Drug concn.	1/40,000	1/10,000				1/10,000	
	Effect on spontaneous activity and tone	0	0				0	
	Effect of combining with light irradiation		0					
	Effect on response to histamine, acetylcholine, adrenaline						0	
Guinea-pig ileum	Drug concn.	1/20,000	1/40–10,000	1/10,000	1/20,000	1/20,000	1/8,000	1/20,000
	Effect on spontaneous activity and tone	0	Slight + twice only	0	+ twice only	0	—	+ (histamine contaminant)
	Effect on response to acetylcholine	0					—	
	Effect on response to 5-OH tryptamine	0		0	0	0		
	Effect on response to histamine		+ (50%) once only				—	

0 = No effect; + = increase; — = decrease.

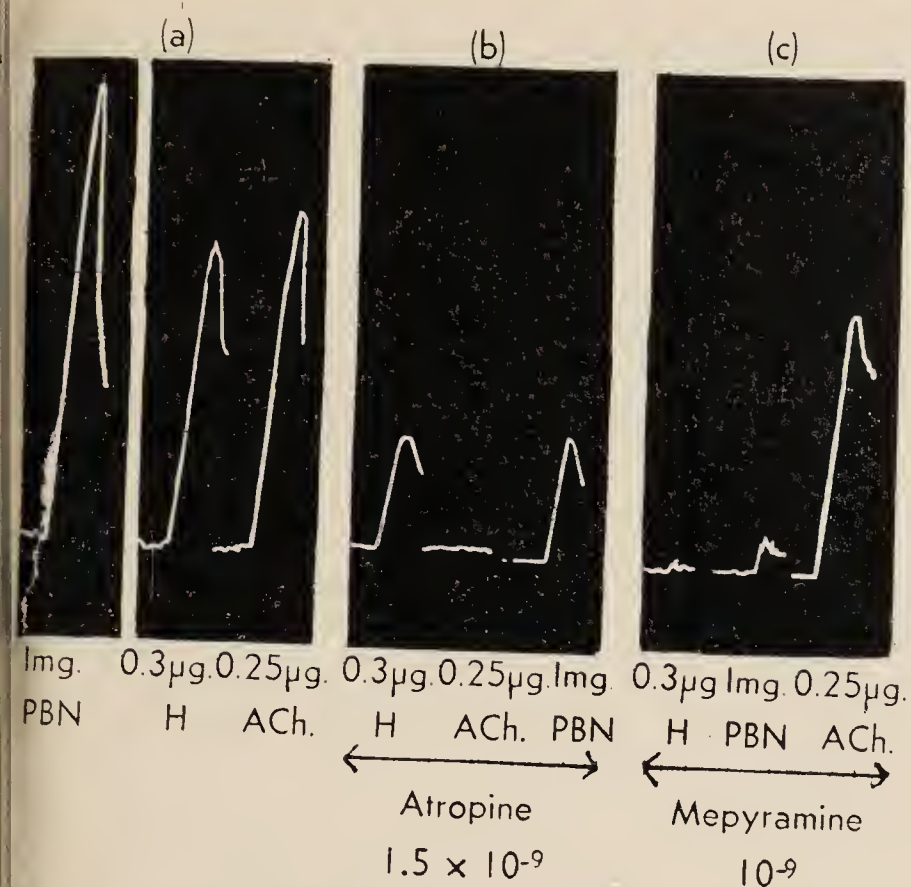


Fig. 2.—Isolated guinea-pig ileum. Response to 1 mg. porphobilin, 0.3 μg. histamine and 0.25 μg. acetylcholine (a) normally, (b) in presence of 1.5×10^{-9} atropine, (c) in presence of 10^{-9} mepyramine.

ically active substance is excreted, a sterile specimen of urine from a patient suffering a moderately severe attack of acute porphyria (with hypertension and abdominal pain) was infused into an anaesthetized cat at a rate of approximately 4 ml./min. for 15 min. This by itself produced no effect on blood pressure or respiration; and its effect on the responses to adrenaline, noradrenaline, histamine, acetylcholine, and nicotine was indistinguishable from that of a specimen of normal urine.

DISCUSSION

Despite a great deal of research on acute porphyria the mechanism of the production of symptoms remains obscure. Recent work has tended to minimize the possible direct influence of the porphyrins (Waldenström, 1939) and to emphasize the importance of the pathological features of patchy melanin change observed in the peripheral and autonomic nerves (Denny-Brown and Sciarra, 1945). These authors considered that the changes might be caused by an intermittent ischaemia, probably due to a circulating vasoconstrictor substance. Following this, Wehrmacher (1952) reported clinical improvement in acute porphyria with the use of ganglion-blocking agents. A search for some such vasoconstrictor substance, which might be present only in active cases of acute porphyria, could be a reasonable approach to the problem. The pharmacological testing of the known and readily purified excretion products was clearly necessary. It would be unlikely that uroporphyrin

and coproporphyrin could fulfil this role, since these are excreted in increased amounts in both congenital porphyria (as the series I isomers), and in porphyria cutanea tarda (as the series I and III isomers) where skin photosensitivity may be the only symptom. Porphobilinogen, however, is always excreted in the urine in attacks of acute porphyria and in those phases of porphyria cutanea tarda where acute symptoms are superimposed on the cutaneous syndrome. For this reason porphobilinogen or some closely related substance has been strongly suspected of being the *materia peccans* of acute porphyria (Lowry *et al.*, 1950).

Our experiments have failed to show that either the porphyrins or porphobilinogen have any significant pharmacological action. The initial animal experiments, in which porphobilinogen appeared to potentiate the blood pressure responses of the cat to adrenaline and noradrenaline, could not be repeated. These interesting results cannot be explained, although it is just possible that there may be an individual tissue and animal sensitivity to such drugs. Their inactivity in our hands is at variance with the results of some previous investigators. The difficulty of isolating porphyrins from biological materials, such as urine, in a state of purity that will guarantee freedom from possible histamine contamination, is very great. Our experience suggests that the contradictory results obtained by previous workers may possibly have been due to histamine contamination.

The results of intravenous injection of porphobilinogen into a rabbit confirm Prunty's (1945) findings, but contradict those of Waldenström and Wendt (1939), who found porphobilinogen in the urine of a rabbit into which they had previously injected the partially purified substance (amount used unknown).

Further work has tended to substantiate the absence of pharmacological activity of the porphyrins and porphobilinogen. An "experimental porphyria" or disturbance of porphyrin metabolism has been produced in rabbits by the non-hypnotic substance allyl isopropyl acetamide (Goldberg, 1953); very large quantities of uroporphyrin and porphobilinogen were excreted—in one animal for as long as three weeks—without obvious pharmacological effect. Apart from constipation, there is no evidence that the state of these rabbits compared with the clinical state of human acute porphyria, although the animals excreted proportionately greater quantities of the substances. Falk, Dresel, and Rimington (1953) have shown that porphobilinogen is a porphyrin precursor in a tissue system. This emphasizes that in porphobilinogen

we are probably dealing with a physiological substance. While our experiments therefore do not rule out the possibility that an unidentified vasoconstrictor substance may be produced in acute porphyria, or that the known excretion products may exert a pressor action by a mechanism at present unknown, they render these suggestions unlikely.

SUMMARY

1. The porphobilinogen and the porphyrins usually excreted in acute porphyria, as well as haematoporphyrin and porphobilin, have been tested pharmacologically.

2. Apart from slight and variable action of uroporphyrin I, coproporphyrin I, and porphobilin, these substances show no pharmacological action.

3. The significance of the results in relation to the symptoms of acute porphyria is discussed.

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